Department of Agriculture, Trade and Consumer Protection
Division of Agricultural Development
Agricultural Development & Diversification Program (ADD)
Grant Project Final Report

Contract Number: 20017

Grant Project Title: **Application of DNA Testing Technology to Achieve Genetic Improvement of Dairy Cow Health and Fertility**

Amount of Funding Awarded: 38,000

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Please use the following questions as a guide for writing your grant project final report. In your final report, please answer each question as it relates to your grant project.

1) What was the original intent of the grant?

The intent of the grant was to identify specific genes that influence dairy cows' susceptibility to ketosis, mastitis, displaced abomasum, metritis, and/or lameness – all costly diseases in the dairy industry. Next, we will develop DNA tests for susceptibility to the aforementioned disorders, which will provide the framework for a marker-assisted selection program for improvement of health traits in dairy cattle. This grant was applied for to obtain DNA samples, extract DNA, and analyze the effect of specific genes on susceptibility of disease in dairy cattle. If disease resistance can be accomplished partly through genetic selection, the Wisconsin Dairy industry stands to save millions of dollars in disease treatment and cattle death costs. This project is part of a growing trend to have more information available for selection of genetics in dairy cattle. Traditionally the only selection could be done on conformation and production, but more recently traits directly affecting longevity and health have become available, but they generally are not measured until a cow leaves the herd, and therefore are not timely. This project aimed at identifying genes that help predict longevity characteristics early in life.

2) What steps did you take to reach your goal?

DNA samples on over 15,000 animals were collected, as part of this project and part of a broader initiative at Alta Genetics. The collection of DNA worked ok, however was a very manual process. For broader objectives at Alta Genetics, a DNA collection tagging device is being considered to collect DNA at the time the animal is identified. This would greatly reduce staff time required for DNA collection. Also, hair follicles appear to be inferior to some other types of DNA (tissue or blood), as some samples invariably are not useable due to a lack of DNA – again the DNA tagging device would have solved this issue. Even though there were a number of challenges with DNA collection and extraction, these were known going into the project, and they were overcome to make a very useful project. The collaboration with the University worked well, however a number of delays made the overall project take more time than originally planned. The goal of determining certain genes that could be selected for to improve disease resistance in dairy cattle, and other areas of the genome to focus future research on were realized.

3) The results from the project are many, and will lead us in the correct direction for both genetic selection, as well as future research. 1) Four chromosomal regions were identified for research – specifically on chromosomes 2, 5, 6, and 14. 3 of the 4 regions identified, had polymorphisms, and 2 of those SNP's can potentially lead to selection for more disease resistant cattle. DNA tests to test cattle for these genes have been developed and can be done on any animals we wish to use as parents for future offspring.

4) What conclusions can you make base on project work the analysis of collected data?

   Our conclusions are that we can test animals for the "C" allele in the OLR1 gene to get an early prediction of that animals merit for fat and protein yield and fat and protein percentage. The importance of this finding could be large, as the "C" allele is also associated with a reduction in SCS – and indication of mastitis. This is particularly interesting because the relationship between production (fat & protein yield) and SCS is generally undesirable (more yield = decreased mastitis resistance), however this particular finding could lead to a way to reverse this trend, as the "C" allele is associated with a desirable relationship between yield and SCS (more yield = increased mastitis resistance). Other diseases could also be affected by this allele, and will be part of a broader project, which collects even more DNA and more phenotypic information to improve the power of the test.

5) What do you plan to do in the future as a result of this project?

   We may begin testing young bulls for the "C" allele, as we continue to collect more data to determine its impact on other diseases. Furthermore, the data for longevity of these cattle is not complete (many of the animals are still in production) – therefore we will be able to analyze the effect of this allele as we get more culling information in on the daughters that were DNA tested. We will also continue testing for other unique genes in these chromosomal regions, to see if we can improve the accuracy of prediction by finding more genes that have a positive impact on disease resistance.

6) What information or additional resources are needed to commercially develop this enterprise?

   Time is needed to collect both more DNA and more phenotypes for these diseases. Also, a larger project has been funded by the USDA (National Research Institute grant) to identify test for 10,000 SNP's across the complete genome. The progress of this project helped us decide to support that project. Those results can be combined with the results found here to determine the effectiveness of SNP testing to improve early predictions of genetic merit. Specifically, the phenotypes collected in this project will be vital to the success of selection for specific genes for disease resistance.

7) How should the agricultural industry use the results from your grant project?

   Anyone could test an animal at a young age for the "C" allele to determine if she was susceptible to mastitis, however the results of this research will be part of a broader initiative that will help us (AI organizations) select for animals that are healthier and easier to maintain throughout their life to help support sustainable farming in Wisconsin through healthier, longer-lasting cattle.

Project Title: **Application of DNA Testing Technology to Achieve Genetic Improvement of Dairy Cow Health and Fertility**

The objective of this proposal was to identify quantitative trait loci (QTL) affecting health traits in Holstein cattle using a candidate gene approach and to subsequently implement these DNA tests in a marker-assisted selection program that can improve the health of Wisconsin's dairy herd. To accomplishment this objective, our plan included following steps:

Step 1: Identification of promising chromosomal regions. Chromosomal regions that are likely to harbor QTL that influence dairy cow health and fertility will be identified by reviewing the findings of recently published genome scans. In the past five years, eight independent studies have identified statistically significant associations between genotypes in regions of 15 bovine chromosomes and somatic cell count, clinical mastitis, or length of productive life. We will identify promising regions of 3-5 chromosomes that have been implicated in multiple, independent studies, as these are most likely to contain important QTL affecting dairy cow health and fertility.

Accomplishments: Based on the analysis of different QTL studies, we have identified four chromosomal regions that are likely to affect milk production and health traits in dairy cattle. These chromosomal region were identified on chromosomes 2, 5, 6, and 14.

Step 2: Identification of potential candidate genes in QTL regions. Because vast public and private resources have been dedicated to genetic research (particularly health-related research) in humans and laboratory species, the functions of many specific genes in mammals have been studied in great detail. Furthermore, the genomes of humans, mice, and dairy cattle are quite similar. Numerous genes have been physically mapped to chromosomal regions in humans and laboratory species, and corresponding regions in cattle and other livestock species can be identified. We will use information from such studies to identify individual genes within promising chromosomal regions (from step 1) that are likely to influence the health and fertility of dairy cattle.

Accomplishments: In dairy cattle and other livestock species, there is increasing interest in using a positional candidate gene approach for the identification of the actual genes that control economically important traits. These genes can be exploited by marker assisted selection for genetic improvement within a breeding nucleus or commercial population; or by marker assisted introgression for transferring desired alleles from a resource population to a commercial population. Currently, genes are identified as candidates to be the quantitative trait genes based on results of previous linkage mapping studies and on comparative biological functions in the same or other species.
In this study, provisional candidate genes were chosen based on possible functions related to production and health traits (functional candidate gene approach) and based on position of these genes in QTL regions (positional candidate gene approach). **STAT1** gene was chosen on **chromosome 2** based on position and function. **OLR1** was chosen on **chromosome 5** using the positional and functional approaches. **OPN** and **PPARGC1A** were chosen on **chromosome 6** using positional candidate gene approach. **TG** was chosen on **chromosome 14** based on the positional approach.

Step 3: Identification of DNA sequence variation between animals in these candidate genes. Modern molecular genetic tools can be used to readily identify single nucleotide polymorphisms (**SNP**) within known, functional genes. This sequence variation between individual animals can lead to differences in the enzymes or proteins produced which, in turn, can influence the animal's health or fertility. We will use published information from Genbank to detect SNP variation within the candidate genes chosen in step (2), using DNA from cows in Alta Genetics' cooperator herds.

Accomplishments:

Polymorphism detection in STAT1 gene: In order to detect single nucleotide polymorphisms (SNPs) in the *STAT1* gene (GenBank accession number AW289395), DNA pools were constructed from 220 bovine samples and amplified with the primers STATF (position 12-31): 5'-GCCTCAAGTTTGCCAGTGGC-3` and STATR (position 325-306): 5'-GGCTCCCTTGATAGAACTGT-3'. Amplification was performed in a 25 µl reaction volume, which included 50 ng genomic DNA, 50 ng each primer, 200 µM each dNTP, 2.5 µl 10X PCR buffer (Promega, Madison, WI), and 0.3 u Taq DNA polymerase (Promega). The temperature cycles were as follows: 95^{o}C for 5 min, followed by 30 cycles of 94^{o}C for 45 s, touchdown annealing from 65- 50^{o}C for 45 s (-2^{o}C/cycle), 72^{o}C for 45 s, and a final extension at 72^{o}C for 7 min. PCR products of the pooled DNA samples were sequenced and SNPs were identified by visually inspecting sequence traces. Using the pooled genomic DNA sequencing approach, a **SNP (C/T) at position 213** was identified.

Polymorphism in OLR1 gene: Direct sequencing of pooled RT-PCR products for the total coding sequence of *OLR1* revealed 2 SNP in exon 4 at positions 7160 (C/T) and 7161 (A/G). The SNP 7160 is a missense mutation in which threonine is replaced by methionine, and SNP 7161 is a synonymous substitution. Direct sequencing of genomic DNA at intron 4 revealed 5 SNP, at positions 7278, 7381, 7409, 7438, and 7512. Direct sequencing of the 3` UTR (genomic DNA) of *OLR1* revealed one SNP (A/C) at position 8232. Four intragenic haplotypes comprising positions 7160, 7161, 7278, 7381, 7409, 7438, 7512, and 8232 were inferred in a sample of 633 individuals from the Holstein population.

Polymorphism of OPN gene: In order to detect single nucleotide polymorphisms in *OPN*, different sets of primers were designed to amplify genomic sequences of the gene. Only one single nucleotide polymorphism (SNP) was identified in intron 4 (GenBank accession number NW_255516) using the primers OPNF: GCAAATCAGAAGTGTGATAGAC and OPNR: CCAAGCCAAACGTATGAGTT.

Step 4: Collection and analysis of phenotype data. A key aspect of this study is the availability of data regarding the incidence of health and fertility disorders. By mid 2005, approximately 60 Alta Genetics bulls will each have 100-120 milking daughters in cooperator herds. These cows, which have detailed health and fertility data, will serve as the study population in our project, and we will genotype selected (i.e., the best and worst 10% of) cows from this population for the SNP variants described in step (3).

Accomplishments: Phenotypes on 5,148 daughters of project sires were collected as a subset of phenotypes that were collected on a total of 128,947 cows. Only the top 10% (about 500 samples) and the bottom 10% (about 500 samples) for phenotypic expression of each trait are useful for association of individual genotypes with disease. Therefore, when collection of DNA is completed prior to the collection of phenotypes, much more DNA must be collected than is ultimately used. The phenotypes were collected directly from on-farm computer management systems, which is very unique to this project. Traditionally, phenotypic information has only been collected on a large scale through DHI sources, however DHI does not collect data on diseases, and therefore the collection of data directly from the farms was necessary. This also proves that collection of data can be accomplished outside of

traditional data sources if some prior planning is done (since we desired data only from our progeny test herds, and could limit the number of our progeny test herds to 175, data collection could be completed directly at the farm level). Data on every "event" in a cow's life was recorded and collected from sickness to pregnancy to death (these included, but were not limited to: milk fever, DA, mastitis, cystic ovaries, metritis, retained placenta, lameness), the process and format of the data collected from the farm will serve as the basis for future collection of this information, which will be continued within the Alta Genetics system.

Step 5: Association of SNP variation with health and fertility traits. The final step is to detect statistically significant associations between health and fertility traits and individual SNP variants within our candidate genes. We will use modern statistical genetic tools to investigate differences in the frequency of specific SNP genotypes between subsets of the best and worst animals for each of the aforementioned health and fertility traits, and we will do this for each of the 3-5 candidate genes.

Accomplishments: In a previous study we have shown that OLR1 gene was associated with milk composition traits in a Holstein cattle population. Haplotype analysis showed that one of the haplotypes was associated with a significant increase in fat yield (P = 0.0022) and fat percentage (P = 0.0066). Single SNP analysis showed that allele C of SNP 8232 (in the 3` UTR) had significant effects on fat yield ($P$ = 0.0005) and fat percentage ($P$ = 0.0033) whereas SNP 7160 and 7161 (in exon 4) had no significant effects. Both single SNP and haplotype analysis indicated that SNP 8232 in the 3`UTR is associated with milk fat yield and percentage and it may be in linkage disequilibrium with the functional polymorphism.
In this study, we have genotyped about 1000 cows from ALTA Genetics population. Results show that OLR1 is associated with SCS, fat, and protein traits which is consistent with our previous results on the CDDR Holstein population. However, because of technical problems in extracting DNA from hair samples, many genotypes were missing and the results need more validation.
It worth noting that other genes, in addition to those mentioned, showed significant polymorphism in ALTA population and could be tested for association with production and health traits, but this analysis focused on the 4 regions identified.

In previous studies we have shown that STAT1 was associated with milk composition and health traits in two Holstein independent populations. For the Cooperative Dairy DNA Repository, allele C was associated with significant increases in milk fat and protein percentages. For the University of Wisconsin population, genotypes CC and CT were associated with significant increases in milk, fat, and protein yields. This gene will be analyzed soon with ALTA Holstein population for disease traits to determine if the general undesirable relationship between production and health traits is broken (and a favorable relationship can be detected) for those animals with the CC or CT genotype.

Overall conclusion: There are many interesting areas of the genome that can be tested. Continued collection of DNA on progeny test daughters (using a superior method), and continued transmission of phenotypic data for disease will be necessary to validate other SNP's and their effect on the health of Holstein cattle. The scope of the bigger goal (genetic selection for healthier cows) has no end, but this project provides the framework for collection of phenotypes, and has created a long-term partnership between Alta Genetics and University of Wisconsin.